Clin Exp Immunol 1999; **115**:477–484

CD4 depletion in HIV-infected haemophilia patients is associated with rapid clearance of immune complex-coated $CD4^+$ lymphocytes

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(Accepted for publication 4 December 1998)

SUMMARY

The predominant immunological finding in HIV^+ haemophilia patients is a decrease of $CD4^+$ lymphocytes during progression of the disease. Depletion of $CD4^+$ lymphocytes is paralleled by an increase in the proportion of immune complex-coated $CD4^+$ cells. We examined the hypothesis that the formation of immune complexes on $CD4^+$ lymphocytes is followed by rapid clearance of immune complex-coated $CD4^+$ lymphocytes from the circulation. In this study, the relationship of relative to absolute numbers of immune complex-loaded $CD4^+$ blood lymphocytes and their association with viral load were studied. Two measurements of relative and absolute numbers of gp120-, IgG- and/or IgM-loaded $CD4^+$ lymphocytes were analysed in HIV^+ and HIV^- haemophilia patients, with a median interval of approx. 3 years. Immune complexes on $CD4^+$ lymphocytes were determined using double-fluorescence flow cytometry and whole blood samples. Viral load was assessed using NASBA and Nuclisens kits. Whereas the proportion of immune complex-coated $CD4^+$ lymphocytes increased with progression of the disease, absolute numbers of immune complex-coated $CD4^+$ lymphocytes in the blood were consistently low. Relative increases of immune complex-coated $CD4^+$ blood lymphocytes were significantly associated with decreases of absolute numbers of circulating $CD4^+$ lymphocytes. The gp120 load on $CD4^+$ blood lymphocytes increased in parallel with the viral load in the blood. These results indicate that immune complex-coated $CD4^+$ lymphocytes are rapidly cleared from the circulation, suggesting that $CD4^+$ reactive autoantibodies and immune complexes are relevant factors in the pathogenesis of AIDS. Relative increases of immune complex-positive cells seem to be a consequence of both an increasing retroviral activity as well as a stronger loading with immune complexes of the reduced number of $CD4^+$ cells remaining during the process of CD4 depletion. The two mechanisms seem to enhance each other and contribute to the progressive CD4 decrease during the course of the disease.

Keywords viral load autoimmunity $CD4^+$ lymphocyte count immune complex load HIV

INTRODUCTION

Most HIV-infected patients demonstrate a progressive depletion of $CD4^+$ lymphocytes and a consistent rise in plasma viral load [1,2]. The $CD4^+$ cell decline is closely associated with the immune complex load on $CD4^+$ lymphocytes and the induction of autoantibodies against the patient's own immunoglobulins [3–6]. Moreover, anti-IgG autoantibodies in HIV-infected haemophilia patients were also associated with thrombocytopenia [7]. During the course of the disease $CD4^+$ lymphocytes are sequentially coated with IgM, IgM/IgG and IgM/IgG/gp120 complexes [8]. Autoantibodies are detected on $CD4^+$ and $CD8^+$ lymphocytes. Only autoantibodies on $CD4^+$ lymphocytes are significantly associated with the depletion of $CD4^+$ and also $CD8^+$ lymphocytes, whereas $CD8^+$ lymphocyte-reactive antibodies are not [9]. Another difference between both types of antibodies is the high frequency of healthy controls with antibodies on $CD8^+$ lymphocytes, especially IgM autoantibodies, in contrast to the few healthy individuals with IgM autoantibodies on $CD4^+$ lymphocytes. Moreover, there is no healthy individual with IgG on $CD4^+$ cells [9].

Differences in the binding of the antibodies and immune complexes to the corresponding lymphocyte subsets, i.e. Fc receptor binding to $CD8^+$ cells *versus* specific binding to $CD4^+$ lymphocytes, might be a plausible explanation for this phenomenon. Our data agree with studies of Nebe *et al*., who reported that

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immunoglobulins on $CD4^+$ and $CD8^+$ lymphocytes of non-haemophilia patients were significantly associated with $CD4^+$ cell decreases presumably induced in part by phagocytosis [10]. The rate of lymphocyte ingestion by monocyte-derived macrophages, analysed by microscopy *ex vivo*, increased significantly with the amount of antibody per T cell [10]. The increasing percentage of circulating $CD4^+gp120^+$ lymphocytes appearing during the course of the disease might be due to (i) the attachment of an increasing number of circulating virions to the $CD4^+$ cell surface, which (ii) in turn induce the release of increasing amounts of soluble gp120 that attaches to $CD4^+$ cells; (iii) an increasing proportion of productively infected $CD4^+$ lymphocytes expressing gp120 on the cell membrane; (iv) the increasing release of $gp120^+CD4^+$ lymphocytes from destroyed lymph nodes; (v) the defective filter function of the destroyed lymph nodes for the $CD4^+gp120^+$ lymphocytes; and (vi) an increasing immune complex concentration on circulating $CD4^+$ lymphocytes due to the decreasing absolute counts of $CD4^+$ lymphocytes in the blood [11].

Based on our data we developed the following hypothesis about the contribution of autoimmune mechanisms to the pathogenesis of AIDS. HIV infection induces the release of cytokines and soluble cytokine receptors [12]. During progression of the disease, Th2 cytokine patterns are detectable which are capable of inducing B cell activation [13]. We believe that the increased cytokine release and the elevated serum levels of circulating cytokines serve as the second signal for B cell activation and facilitate a broad autoantibody response when autoreactive B cells are stimulated by their corresponding antigens (first signal). First-signal stimuli are provided by structural homologies of virus peptides with autoantigens as well as autoantigens unmasked during protein degradation after cell death. As a consequence, autoantibodies against several cellular and humoral autoantigens are formed [6,7,14–21]. In addition, gp120 of HIV activates the complement system directly [22,23]. The immune complexes attach to surfaces of HIV-infected as well as HIV-uninfected cells that will be eliminated from the circulation by antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis and apoptosis.

To understand the role of immune complexes on $CD4^+$ lymphocytes in the pathogenesis of AIDS it is important to know whether immune complex-coated $CD4^+$ lymphocytes are rapidly cleared from the circulation, or if these cells accumulate in the blood during progression of the disease. One would interpret accumulating immune complex-coated $CD4^+$ cells as an epiphenomenon of disease progression, whereas rapid clearance from the circulation, especially during periods of relative $CD4^+$ increases in the blood, would point towards an immunopathogenic relevance of immune complexes for the depletion of $CD4^+$ lymphocytes.

MATERIALS AND METHODS

The first and the latest autoantibody measurement in 81 HIV^+ and nine HIV^- haemophilia patients was analysed (median interval between the two determinations: HIV^+ 1298 days; HIV^- 1191 days). The patients were infected with HIV in the early 1980s and they were part of an ongoing long-term study of immunological parameters in haemophilia patients [8]. Most of the patients were treated with AZT or a combination of two nucleoside analogues, and they were converted to triple-drug treatment, consisting of two nucleoside analogues and a reverse transcriptase inhibitor during the second half of 1996. Eight HIV^+ patients refused treatment with anti-retroviral drugs.

Determination of HIV-1 RNA copies in 100 μl plasma

HIV-1 RNA was measured using the NASBA HIV1 RNA QT-kit (Organon Teknika, Heidelberg, Germany). According to the manufacturer's instructions, HIV-1 RNA was quantified using a 100-μl plasma sample. Quantification is based on coamplification of HIV-1 wild-type RNA with three internal calibrators (QA, QB, QC) differing only by sequence randomization of a 20 nucleotide fragment of the wild-type RNA, thereby ensuring efficiency of isolation and amplification. RNA is amplified directly and selectively based on the activity of reverse transcriptase, RNaseH and T7RNA polymerase. The quantity of the amplified product was measured by electron-chemiluminescence using the automated NASBA QR System. The sensitivity of the assay is >4000 copies using samples of 100 μl plasma. Since April 1997 the sensitivity of the test was increased to >80 HIV-1 RNA copies/ml using the NucliSens QT Kit (Organon Teknika) and samples of 1 ml patient plasma. Sera of patients with <4000 HIV-1 RNA copies/ml were retested with the NucliSens QT Kit. There was no patient in this study whose viral load at the first or second measurement was below the detection limit of the assay.

Determination of immunoglobulin, IgG, IgM, C3d and gp120 on $CD4^+$ T lymphocytes

The proportion of immunoglobulin-positive $CD4^+$ cells in the peripheral blood was determined using double fluorescence flow cytometry as described in detail previously [24]. Briefly, 100 μl whole blood were incubated with 10 μl anti-CD3 (OKT3, all T lymphocytes; Ortho, Raritan, NJ), anti-CD4 (OKT4, helper/inducer T lymphocytes; Ortho) or anti-CD8 (OKT8, suppressor/cytotoxic T lymphocytes; Ortho) MoAb for 30 min at 4°C. Erythrocytes were lysed by the addition of NH_4Cl solution for 15 min, the cells were washed with PBS, and 50 μl PE-conjugated goat anti-mouse immunoglobulin (Dianova, Hamburg, Germany) were added, together with 50 μl FITC-labelled goat anti-human immunoglobulin (Medac, Hamburg, Germany), goat anti-human IgG (Tago, Burlingame, CA), goat anti-human IgM (Medac), rabbit anti-human C3d (Dakopatts, Hamburg, Germany), or 10 μl sheep anti-gp120 (Biochrom, Berlin, Germany). Sheep anti-gp120 was used undiluted, the other antibodies were diluted 1:40. The cells were incubated for another 30 min at 4°C, washed and analysed by flow cytometry (FACScan; Becton Dickinson, Sunnyvale, CA). The gate setting for background staining was adjusted to <5% $CD3^+IgG^+$ control lymphocytes and this gate was used for all subsequent analyses. Absolute counts of $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes were determined as described previously [3].

Definition of cut-offs for the discrimination between immune complex-positive and immune complex-negative $CD4^+$ lymphocytes

A test result was defined as autoantibody-positive if >30% of $CD4^+$ blood lymphocytes were stained with a particular antibody in the double fluorescence experiment. As reported previously, at a 30% cut-off, 218 of 223 healthy controls (laboratory staff and voluntary blood donors) showed no immune complexes at all and the remaining five controls showed IgM but no IgG on $CD4^+$ lymphocytes [8]. Of the 37 HIV^- haemophilia patients, 33 showed no evidence of immune complexes and three had IgM but no IgG on their $CD4^+$ lymphocytes [8].

Table 1. Relative and absolute numbers of gp120-, IgG- and IgM-coated $CD4^+$ lymphocytes and viral load in 71 HIV^+ haemophilia patients with ≤ or > 30% immune complex-coated $CD4^+$ lymphocytes in the blood

	CD4gp120%	CD4IgG%	CD4IgM%	CD4/μl	CD4gp120/μl	CD4IgG/μl	CD4IgM/μl	HIV-1 RNA (copies/ml)
CD4gp120%								
> 30% ($n = 24$)	60*****	65*****	85***	29*****	14	13	19***	120000*
≤ 30% ($n = 47$)	11	26	56	251	15	30	99	38000
CD4IgG%								
> 30% ($n = 39$)	33*****	65*****	79*****	65***	21	44***	37	70000
≤ 30% ($n = 32$)	9	12	36	225	13	14	59	48000
CD4IgM%								
> 30% ($n = 60$)	19**	43****	66*****	97**	14	19	46	59000
≤ 30% ($n = 11$)	9	10	16	329	18	15	50	35000

Data are given as medians.
Mann–Whitney-test: > 30% *versus* ≤ 30%: *$P = 0{\cdot}06$; **$P < 0{\cdot}05$; ***$P < 0{\cdot}01$; ****$P < 0{\cdot}001$; *****$P < 0{\cdot}0001$.

Statistical analysis

The Wilcoxon signed rank test and Mann–Whitney test were used for statistical analysis.

RESULTS

Relative and absolute numbers of immune complex-coated $CD4^+$ lymphocytes in the blood

HIV^+ patients were grouped into IgG, IgM or gp120 immune complex-positive and immune complex-negative individuals (Table 1). Patients who were positive (> 30%) for one type of immune complex usually were positive (> 30%) also for the other two types. However, the absolute counts of immune complex-coated $CD4^+$ blood lymphocytes were similar in patients with > 30% or ≤ 30% immune complex-positive lymphocytes, indicating that relative increases of immune complex-coated lymphocytes were rarely associated with absolute increases of these cells in the circulation (Table 1). This was true for $CD4^+IgM^+$ and $CD4^+gp120^+$ blood lymphocytes, whereas $CD4^+IgG^+$ showed both an absolute and a relative increase ($P < 0{\cdot}01$) (Table 1).

Relative numbers of immune complex-coated $CD4^+$ lymphocytes and $CD4^+$ blood lymphocyte counts

HIV^+ haemophilia patients with gp120, IgG or IgM immune complexes on circulating $CD4^+$ lymphocytes had significantly lower absolute $CD4^+$ cell counts than patients without immune complexes (Table 1). Patients with gp120 complexes had a median of 29 $CD4^+$ lymphocytes/μl (compared with 251/μl for gp120-negative individuals; $P < 0{\cdot}0001$), patients with IgG a median of 65/μl (*versus* 225/μl for IgG-negative; $P < 0{\cdot}01$) and patients with IgM a median of 97/μl (*versus* 329/μl for IgM-negative; $P < 0{\cdot}05$).

Relative numbers of immune complex-coated $CD4^+$ lymphocytes and viral load

HIV-1 viral load was associated neither with IgG nor with IgM complexes, but showed a weak association with gp120 complexes on $CD4^+$ blood cells ($P = 0{\cdot}06$) (Table 1).

Relative and absolute numbers of immune complex-coated $CD4^+$ lymphocytes measured twice in 81 HIV^+ and nine HIV^- haemophilia patients

The absolute numbers of immune complex-coated $CD4^+$ blood

Table 2. Relative and absolute numbers of IgG-, IgM- and gp120-coated $CD4^+$ lymphocytes in the blood of 81 HIV^+ and nine HIV^- haemophilia patients

Parameter	HIV^+ patients ($n = 81$), first measurement	HIV^+ patients ($n = 81$), second measurement	HIV^- patients ($n = 9$), first measurement	HIV^- patients ($n = 9$), second measurement
CD4IgG%	18	34*	5	3
CD4IgM%	38	58	13	3*
CD4gp120%	5	16**	4	1
Lymphocytes/μl	1198	1065*	2497	2016
CD4%	16	11**	37	37
CD4/μl	231	107**	780	766
CD4IgG/μl	23	15	61	15
CD4IgM/μl	54	26	100	28*
CD4gp120/μl	7	13*	51	10

Interval between first and second measurement: HIV^+ 1298 days, HIV^- 1191 days.
Data are given as median.
Wilcoxon signed rank test: first *versus* second measurement: *$P < 0{\cdot}01$; **$P < 0{\cdot}0001$.

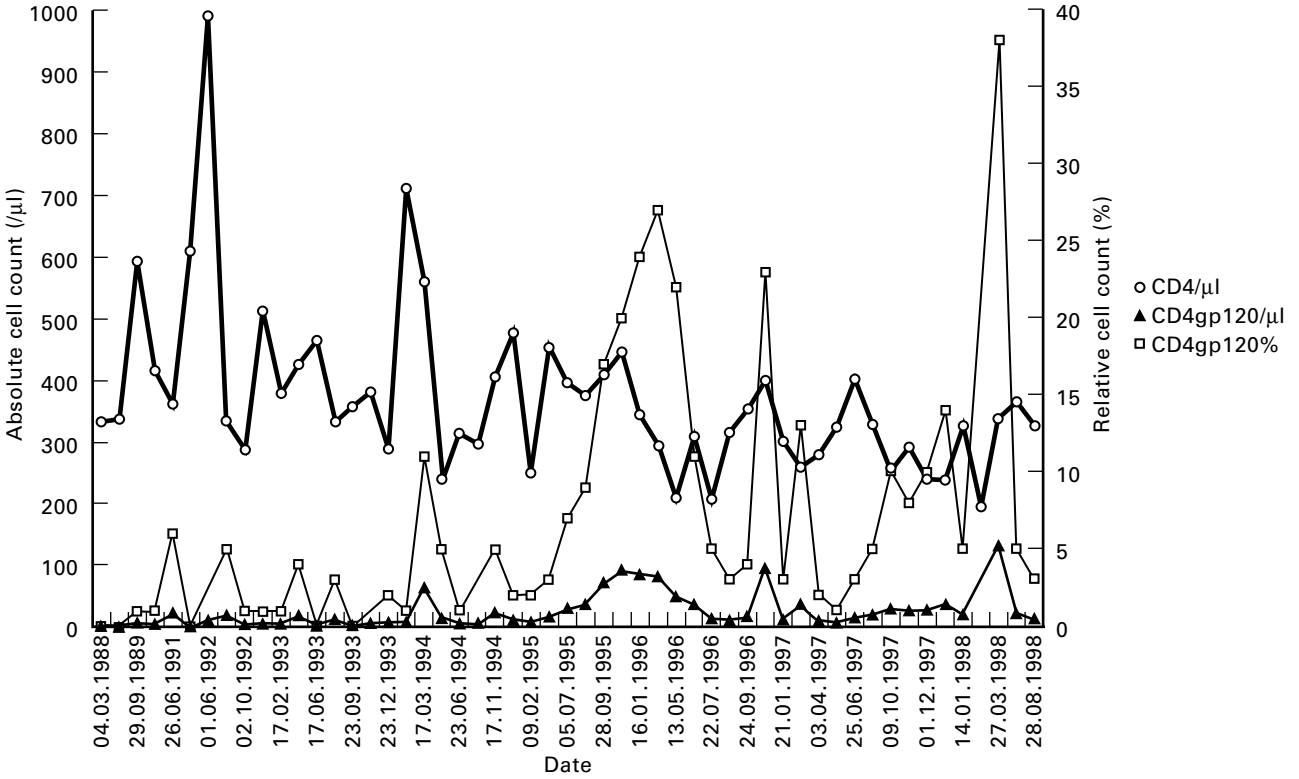

Fig. 1. Patient with low gp120 load on $CD4^+$ blood lymphocytes. $CD4^+$ lymphocytes of patient A decreased from 990/μl in 1992 to 207/μl in 1996. Increases of $CD4^+gp120^+$ lymphocytes were associated with decreases of the $CD4^+$ cell counts. The $CD4^+$ cell decrease in 1995/96 was associated with the second highest relative and absolute rise of $CD4^+gp120^+$ cells during the observation period. The $CD4^+gp120^+$ cells peaked at 27%, the absolute counts of $CD4^+gp120^+$ lymphocytes at 91/μl.

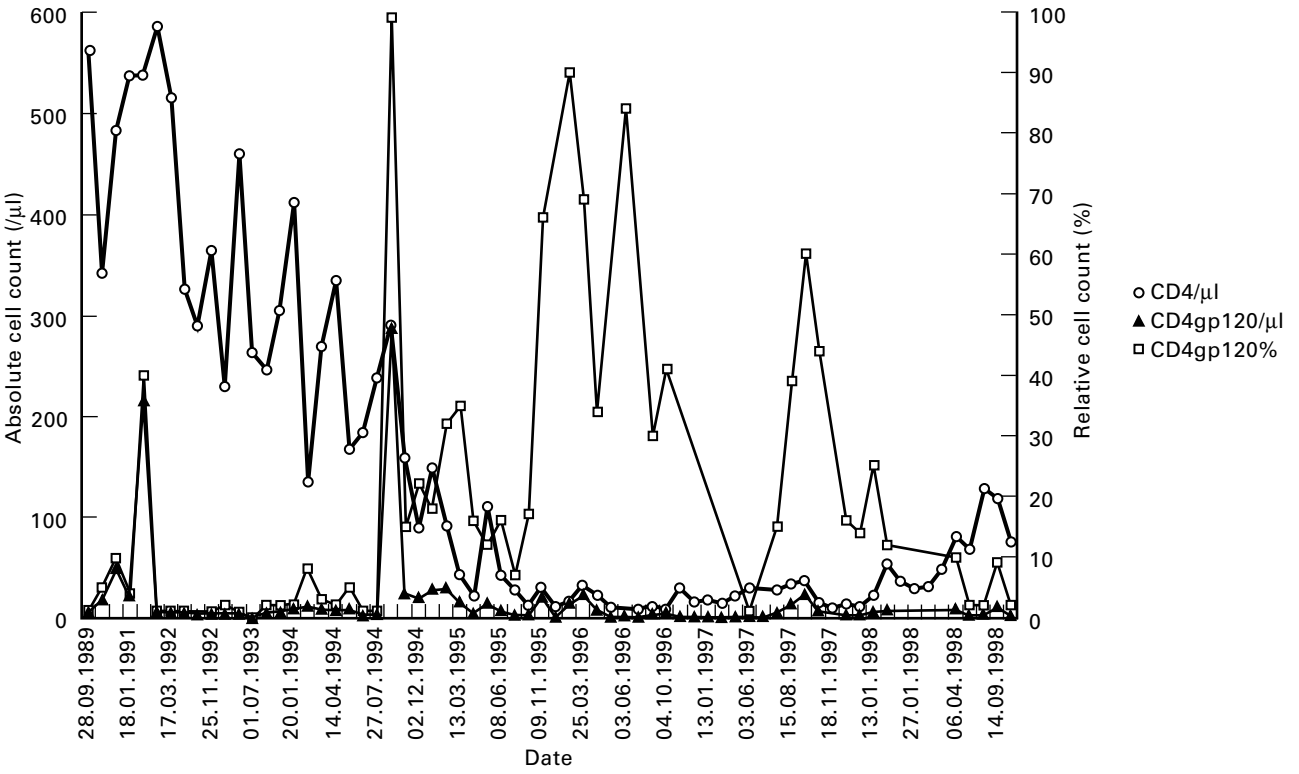

Fig. 2. Patient with high gp120 load on $CD4^+$ blood lymphocytes. $CD4^+$ lymphocytes of patient B decreased from 586/μl to 8/μl during the course of the disease. When $CD4^+$ lymphocytes dropped < 300/μl in 1994, $CD4^+gp120^+$ cells became detectable, with peak increases to 99% during periods of extremely low $CD4^+$ lymphocyte counts. The $CD4^+gp120^+$ lymphocyte counts were < 50/μl with two exceptions in 1991 and 1994.

lymphocytes were stable during the course of the disease (Table 2). The first and the latest measurements (median interval 1298 days) of immune complex-coated $CD4^+$ lymphocytes were analysed in 81 HIV^+ haemophilia patients. Whereas the percentage of IgG- (18% *versus* 34%; $P < 0{\cdot}01$) and gp120- (5% *versus* 16%; $P < 0{\cdot}0001$) coated $CD4^+$ lymphocytes increased significantly during the observation period, rises in absolute cell counts were only found for $CD4^+gp120^+$ lymphocytes (7/μl *versus* 13/μl; $P < 0{\cdot}01$). Total lymphocyte counts as well as relative and absolute numbers of $CD4^+$ lymphocytes decreased significantly (1198 *versus* 1065 lymphocytes/μl, $P < 0{\cdot}01$; 16% *versus* 11% $CD4^+$ lymphocytes, $P < 0{\cdot}0001$; 231 *versus* 107 $CD4^+$ lymphocytes/μl, $P < 0{\cdot}0001$) (Table 2).

Individual patient profiles

Figures 1, 2, 3 and 4 show $CD4^+$ lymphocyte profiles of four HIV^+ haemophilia patients. Immune complex measurements were initiated in 1988/89. Patient A had always low proportions of $CD4^+gp120^+$ blood lymphocytes (< 30%) with one exception in 1998, patient B had increases of $CD4^+gp120^+$ cells to peaks of 99%, patient C showed a decrease of $CD4^+gp120^+$ cells that was associated with a marked increase of $CD4^+$ blood lymphocyte counts, and patient D had strongly fluctuating $CD4^+$ lymphocyte counts with inverse associations of $CD4^+gp120^+$ lymphocytes. The four figures demonstrate that in all four patients, $CD4^+gp120^+$ absolute cell counts did not rise as strongly as the relative proportions of $CD4^+gp120^+$ cells. With very few exceptions, the absolute $CD4^+gp120^+$ lymphocyte count was consistently < 100/μl and often even < 50/μl.

$CD4^+$ lymphocytes of patient A (Fig. 1) decreased from 990/μl in 1992 to 207/μl in 1996. The 11 increases of $CD4^+gp120^+$ lymphocytes were associated with decreases of the $CD4^+$ cell counts. The $CD4^+$ cell decrease in 1995/96 was associated with the second highest relative and absolute rise of $CD4^+gp120^+$ cells during the observation period—however, only 27% of the $CD4^+$ blood lymphocytes were coated with gp120 complexes at that time.

$CD4^+$ lymphocytes of patient B decreased from 586/μl to 8/μl during the course of the disease (Fig. 2). When $CD4^+$ lymphocytes dropped below 300/μl in 1994, $CD4^+gp120^+$ cells became detectable, with peak increases of 99% during periods of extremely low $CD4^+$ lymphocyte counts.

Patient C had 231 $CD4^+$ lymphocytes/μl in 1992, decreasing to five $CD4^+$ cells/μl in 1995/96 and increasing to 113 $CD4^+$ cells/μl in 1996/97 after the initiation of triple-drug treatment (Fig. 3). During the periods of extremely low $CD4^+$ cell numbers the patient had 30–80% $CD4^+gp120^+$ cells. When $CD4^+$ cells increased in 1996 the immune complex-coated $CD4^+$ cells decreased. In 1997 gp120-loaded cells increased again and $CD4^+$ lymphocytes began to fluctuate. The profile of patient C suggests that triple-drug therapy is able to inhibit immune complex formation on $CD4^+$ lymphocytes and that the suppression of immune complex formation contributed to the increase of $CD4^+$ lymphocytes in the patient.

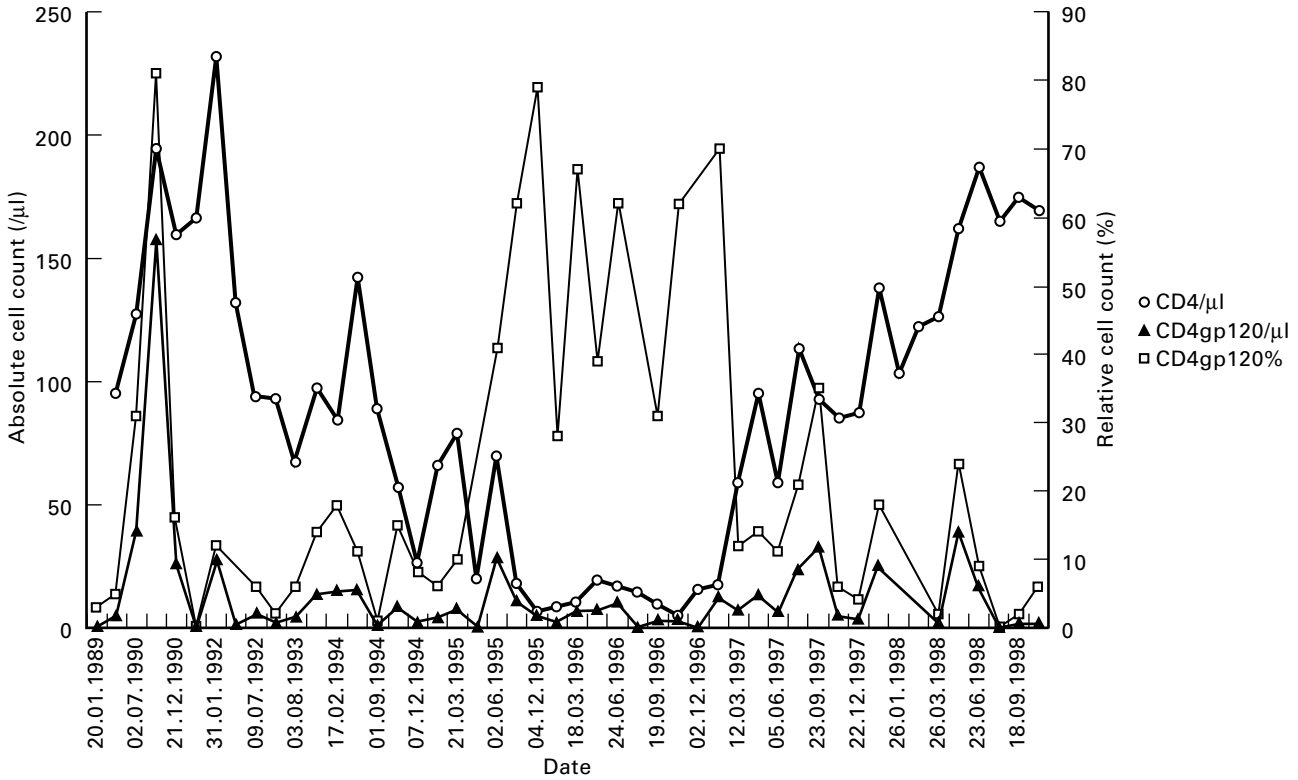

Fig. 3. Patient with high gp120 load on $CD4^+$ blood lymphocytes followed by a decrease of the gp120 load and an increase of $CD4^+$ blood lymphocyte counts. Patient C had 231 $CD4^+$ lymphocytes/μl in 1992. The $CD4^+$ lymphocyte count decreased to five $CD4^+$ cells/μl in 1995/96 and increased to 113 $CD4^+$ cells/μl in 1996/97 after the initiation of triple-drug treatment. During periods of extremely low $CD4^+$ cell numbers the patient had 30–80% $CD4^+gp120^+$ cells. When $CD4^+gp120^+$ cells decreased in 1996/97, $CD4^+$ cells increased again and began to fluctuate during the subsequent period of increasing $CD4^+gp120^+$ lymphocytes. $CD4^+gp120^+$ lymphocytes were stable at levels of < 50/μl with one exception in 1990.

Patient D (Fig. 4) showed strongly fluctuating $CD4^+$ lymphocyte counts during 1989–98, with inverse fluctuations of $CD4^+$ $gp120^+$ lymphocytes. Although the relative numbers of $CD4^+$ $gp120^+$ lymphocytes increased to 48%, $CD4^+$ $gp120^+$ cell counts were consistently $< 100/\mu l$.

Relative and absolute numbers of immune complex-coated $CD4^+$ lymphocytes in HIV^- haemophilia patients

HIV^- haemophilia patients showed low percentages of IgG, IgM and background staining of gp120-coated $CD4^+$ lymphocytes (Table 2). Because of the high absolute lymphocyte counts of $> 2000/\mu l$ at both measurements, the absolute numbers of IgG-, IgM- and gp120-loaded $CD4^+$ cells in HIV^- haemophilia patients were similar to the corresponding values in HIV^+ haemophilia patients.

DISCUSSION

HIV^+ patients show a progressive decline of $CD4^+$ lymphocytes during the course of the disease. Beside the direct cytopathic effect of HIV on $CD4^+$ lymphocytes, apoptosis, metabolic abnormalities, syncytia formation, vβ-deletion, autoreactive cytotoxic T cells, and anti-immunoglobulin autoantibodies as well as immune complex-mediated autoimmune phenomena were reported to play a role in the pathogenesis of CD4 depletion [7,9,17,20,21,25–27].

We reported previously on the formation of immune complexes in HIV^+ patients that reacted with circulating $CD4^+$ lymphocytes, and on a sequential formation of IgM, IgM/IgG, and IgM/IgG/gp120 complexes on $CD4^+$ cells during the course of the disease [8]. The generation of immune complexes increased during periods of enhanced viral activity and led to high proportions of immune complex-coated $CD4^+$ lymphocytes in the blood. Increased percentages of immune complex-loaded $CD4^+$ lymphocytes were associated with decreased $CD4^+$ lymphocyte numbers [8]. The present analysis demonstrates that the absolute numbers of immune complex-coated $CD4^+$ lymphocytes in the blood of HIV^+ patients remained stable at background levels, as defined as the relative and absolute numbers of double-stained $CD4^+$ lymphocytes in blood samples of HIV^- haemophilia patients. The only plausible explanation for this is that immune complex-positive $CD4^+$ lymphocytes were rapidly cleared from the circulation.

All HIV^+ patients had detectable HIV-1 mRNA levels in the blood. Haemophilia patients were infected with HIV nearly 20 years ago. They were treated with several combinations of anti-retroviral drugs. A recently published study demonstrated that pretreated patients did not respond to highly active anti-retroviral treatment (HAART) as well as patients who were initially treated with HAART [28]. However, our very recent measurements of the viral load showed in some patients a trend to HIV-1 mRNA copy levels below the detection limit of the test system. Our experience with a few patients showed that the very low levels of viral load were not stable in all patients but increased again in some patients treated with HAART.

The weak association of gp120 immune complexes on $CD4^+$ blood lymphocytes with increased retroviral activity may be related to (i) increased numbers of circulating virions that were attached to the lymphocyte surface, (ii) increased amounts of free gp120 released from the virions, (iii) increased expression of gp120 molecules on $CD4^+$ lymphocytes during virus budding, (iv) the increasing release of $gp120^+CD4^+$ lymphocytes from

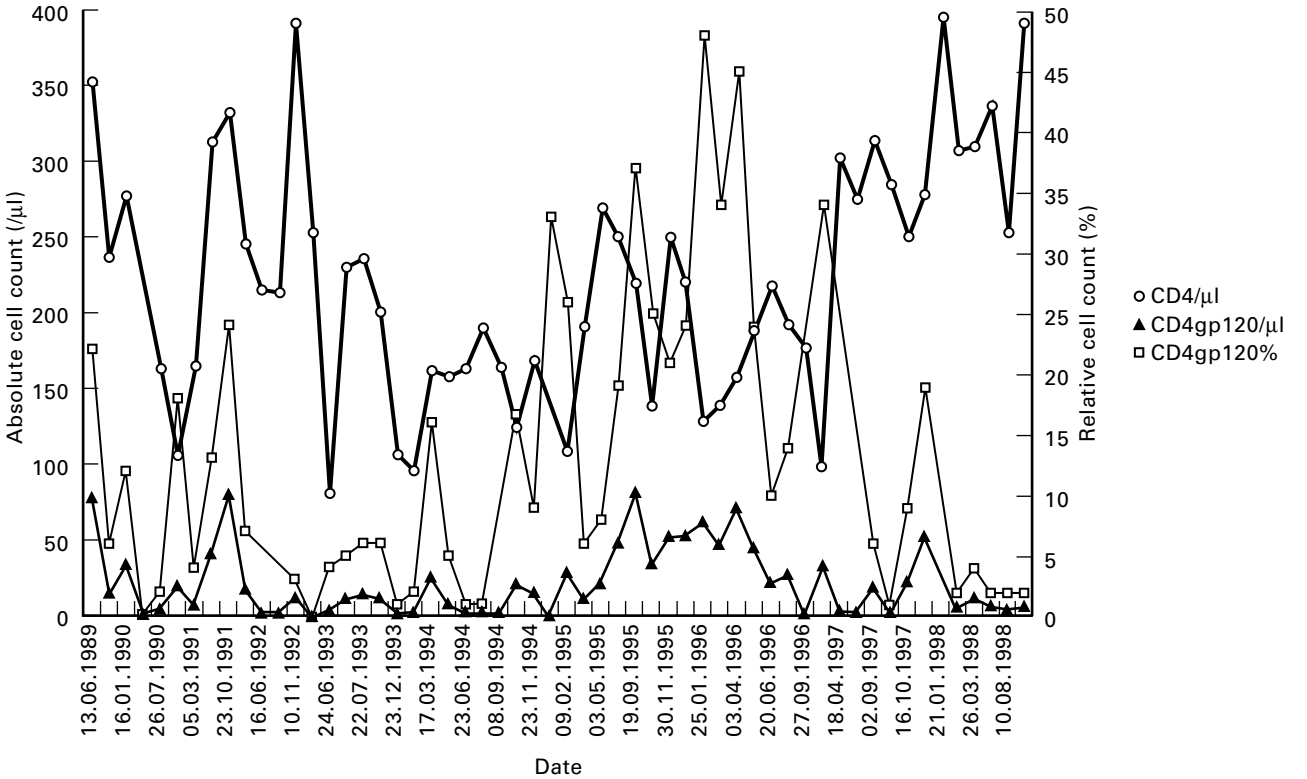

Fig. 4. Patient with strongly fluctuating $CD4^+$ lymphocyte counts and inverse association of $CD4^+$ $gp120^+$ lymphocytes. Patient D showed strongly fluctuating $CD4^+$ lymphocyte counts during 1989–98 with inverse associations of $CD4^+$ $gp120^+$ lymphocytes. Although relative numbers of $CD4^+$ $gp120^+$ lymphocytes increased to 48%, $CD4^+$ $gp120^+$ cell counts were consistently $< 100/\mu l$.

destroyed lymph nodes, (v) the defective filter function of the destroyed lymph nodes for the $CD4^+ gp120^+$ lymphocytes, or (vi) an increasing immune complex concentration on circulating $CD4^+$ lymphocytes due to the decreasing absolute counts of $CD4^+$ lymphocytes in the blood.

The gate setting for background staining was adjusted to < 5% $CD3^+ IgG^+$ control lymphocytes and this gate was used for all subsequent analyses. The median of 5% $CD4^+ gp120^+$ lymphocytes in the blood of HIV^+ and 4% in the blood of HIV^-haemophilia patients in Table 2 refers to background staining for $CD4^+ gp120^+$ lymphocytes in both groups. The gate setting was adjusted to < 5% stained cells to improve the sensitivity for the determination of weakly fluorescing cells. In our previous publications only results of > 30% double-stained cells were considered as immune complex-positive cells [5,8,9]. As shown in Table 2, the percentage of $CD4^+ gp120^+$ cells significantly increased with time in HIV^+ haemophilia patients, whereas it remained at background levels in HIV^-haemophilia patients.

Concerning the specificity of the anti-gp120 polyclonal antibody, it should be emphasized that $CD4^+ gp120^+$ blood lymphocytes above background level were not found in healthy controls, HIV^- haemophilia patients, patients with habitual abortion, pharyngeal cancer, or in patients before or after kidney transplantation as published in previous manuscripts [8,9].

Many HIV^+ and HIV^- haemophilia patients suffer from chronic viral infections such as hepatitis B, cytomegalovirus (CMV), Epstein–Barr virus (EBV), etc., that are able to induce anti-lymphocyte autoantibodies of the IgM type [29–31]. Conceivably, a decrease in IgM-coated $CD4^+$ lymphocytes reflects the application and efficiency of improved treatment protocols against viral infections during the past years.

Relative increases of immune complex-positive cells seem to be a consequence of (i) rising viral activity inducing the formation of immune complexes, as well as (ii) stronger loading of the remaining $CD4^+$ cells with immune complexes as a result of depletion of the target cells for the complexes—the $CD4^+$ lymphocytes themselves. The two mechanisms probably enhance each other and contribute to the progressive CD4 decrease during progression of the disease. Immune complex-coated cells apparently are rapidly cleared from the circulation, probably by ADCC, phagocytosis in lymph nodes and the spleen, and apoptosis [32–41]. Increased ADCC in HIV^+ patients was reported by Tyler *et al*. [42,43], and Lyerly [44,45]. Baumler *et al*. published increased activation of the CD95 (APO-1/Fas) system in T cells from HIV-1-infected children [46]. The accelerated activation-induced cell death of T cells could partially be inhibited by blocking anti-CD95 antibody fragments [46]. Increased phagocytosis of immunoglobulin-coated lymphocytes was reported by Nebe *et al*. [10].

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent technical assistance of Cima Farahmandi, Gaby Schmeckenbecher, and Regina Seemuth.

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